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In re Application of:

Fandke et al.

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For: METHOD AND NUCLEIC ACIDS FOR THE DETECTION OF MICROORGANISMS RELEVANT TO BREWING

PENDING CLAIMS AFTER ENTRY OF PRELIMINARY AMENDMENT

42. Method for the detection of a microorganism relevant to brewing in a sample, which comprises the following steps:

(a) bringing the sample into contact with a combination of at least two first nucleic acid molecules (primers), which hybridise with a region of a microbial nucleic acid conserved in microorganisms relevant to brewing;

(b) amplification of the microbial nucleic acid or a portion thereof to produce at least one amplification fragment;

(c) bringing the amplification fragments obtained in step (b) into contact with at least one second nucleic acid molecule (probe), which specifically hybridises with at least one amplification fragment that comprises a sequence of the microbial nucleic acid specific for all microorganisms relevant to brewing or for one or several families, genera or species of microorganisms relevant to brewing; and

(d) detection of at least one hybrid nucleic acid which consists of an amplification fragment and a second nucleic acid molecule introduced in step (c), whereupon a microorganism relevant to brewing is detected in a sample.

43. Method according to Claim 42, characterised in that as second nucleic acid molecule (probe) at least one nucleic acid molecule, selected from

(i) a nucleic acid with a sequence according to SEQ ID NOS: 1-107 or a fragment thereof at least 10 nucleotides long;

(ii) a nucleic acid which specifically hybridises with a nucleic acid according to (i);

(iii) a nucleic acid which is at least 70% identical with a nucleic acid according to (i) or (ii), and

(iv) a nucleic acid which is complementary to a nucleic acid according to (i) to (iii).

44. Method according to Claim 43, characterised in that as second nucleic acid molecule (probe) at least one nucleic acid molecule with a sequence according to one of SEQ ID NOS: 35-39 or 98-107 is used.

45. Method according to Claim 43, characterised in that as second or further nucleic acid molecule (probe) at least one nucleic acid molecule with a sequence according to one of SEQ ID NOS: 21-34 or SEQ ID NO 73-97 is used.

46. Method according to Claim 42, characterised in that in step (a) a combination of at least two nucleic acid molecules is used, combination being selected from

(i) a nucleic acid with a sequence according to SEQ ID NOS: 1-107 or a fragment thereof at least 10 nucleotides long;

(ii) a nucleic acid which specifically hybridises with a nucleic acid according to (i);

(iii) a nucleic acid which is at least 70% identical with a nucleic acid according to (i) or (ii),

(iv) a nucleic acid which is complementary to a nucleic acid according to (i) to (iii), and

(v) a combination which comprises at least one nucleic acid molecule with a sequence according to one of the SEQ ID NOS: 40-47 and at least one nucleic acid molecule with a sequence according to SEQ ID NOS: 48-54, SEQ ID NOS: 55-59 or SEQ ID NOS: 60-72.

47. Method according to Claim 46, characterised in that as second nucleic acid molecule (probe) at least one nucleic acid molecule according to (i)-(iv) is used.

48. Method according to Claim 47, characterised in that as second nucleic acid molecule (probe) at least one nucleic acid molecule with a sequence according to one of SEQ ID NOS: 35-39 or 98-107 is used.

49. Method according to Claim 47, characterised in that as second or further nucleic acid molecule (probe) at least one nucleic acid molecule with a sequence according to one of SEQ ID NOS: 21-34 or SEQ ID NO 73-97 is used.

50. Method according to Claim 42, characterised in that the amplification comprises a polymerase chain reaction (PCR).

51. Method according to Claim 42, characterised in that the amplification comprises a ligase chain reaction.

52. Method according to Claim 42, characterised in that the amplification comprises an isothermal nucleic acid amplification.

53. Method according to Claim 42, characterised in that the second nucleic acid molecule is modified or labelled to produce a detectable signal, the modification or labelling being selected from (i) radioactive groups, (ii) coloured groups, (iii) fluorescent groups, (iv) groups for immobilisation on a solid phase and (v) groups which allow an indirect or direct reaction, particularly by means of antibodies, antigens, enzymes and/or substances with affinity for enzymes or enzyme complexes.

54. Method according to Claim 42, characterised in that the first nucleic acid molecule and/or the second nucleic acid molecule are at least 10 nucleotides long.

55. Method according to Claim 54, characterized in that the first nucleic acid molecule and/or the second nucleic acid molecule are at least 15-30 nucleotides long.

56. Method according to Claim 42, characterised in that the first nucleic acid molecule and/or the second nucleic acid molecule is modified in that up to 20% of the nucleotides in 10 consecutive nucleotides are replaced by nucleotides which do not naturally occur in bacteria.

57. Method according to Claim 42, characterised in that the conserved region occurs in the genome section which contains the bacterial 23 S and 5 S genes.

58. Nucleic acid molecule as probe and/or primer for the detection of microorganisms relevant to brewing, said nucleic acid molecule being selected from:

(i) a nucleic acid with a sequence according to SEQ ID NOS: 1-107 or a fragment thereof at least 10 nucleotides long;

(ii) a nucleic acid which specifically hybridises with a nucleic acid according to (i);

(iii) a nucleic acid which is at least 70% identical with a nucleic acid according to (i) or (ii), and

(iv) a nucleic acid which is complementary to a nucleic acid according to (i) to (iii).

59. Nucleic acid molecule of Claim 58, wherein the nucleic acid of (i) is at least 15-30 nucleotides long and the nucleic acid of (iii) is at least 90% identical with a nucleic acid according to (i) or (ii).

60. Nucleic acid molecule according to Claim 58, characterised in that it is a DNA or an RNA.

61. Nucleic acid molecule according to Claim 58, characterised in that it is a PNA.

62. Nucleic acid molecule according to Claim 58, characterised in that up to 20% of the nucleotides in 10 consecutive nucleotides are replaced by nucleotides which do not occur naturally in bacteria.

63. Combination of at least two nucleic acid molecules, said combination being selected from:

(1) a combination of at least two nucleic acid molecules according to Claim 58, and

(2) a combination which comprises at least one nucleic acid molecule with a sequence according to one of the SEQ ID NOS: 40-47 and at least one nucleic acid molecule with a sequence according to SEQ ID NOS: 48-54, SEQ ID NOS: 55-59 or SEQ ID NOS: 60-72.